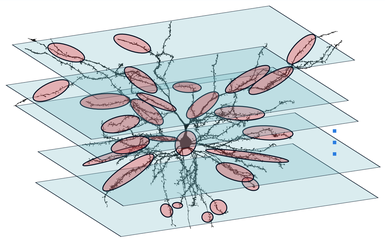
Measuring Many Inputs to a Neuron *in vivo* with High-Speed 2-Photon Imaging

Michael E. Xie[1,2], Adrian Negrean[1], Lucas Kinsey[1], Georg Jaindl[3], Adam S. Charles[2], Karel Svoboda[1], Kaspar Podgorski[1]
1. Allen Institute for Neural Dynamics, 2. Johns Hopkins University, 3. MBF Bioscience

PSTR103.10

BACKGROUND

- **Thousands of inputs** converge on a neuron's dendrites and are **converted into a single output**
- Dendritic trees enable neurons to perform **highly non-linear computations**
- Conventional methods only allow observation of a **very small proportion of inputs** at once, which is **not sufficient to characterize single neuron computation**



*Measuring many inputs to a neuron requires **efficient 3D imaging** with **high spatial & temporal resolution***

METHODS

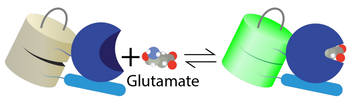
- **Highly-sensitive fluorescent glutamate sensor** (iGluSnFR) in cortical layer 2/3 pyramidal cells
- Image **only in areas of interest** with **no per-ROI access time costs**, using **a novel scan engine** for 2-photon imaging (SLAP2 microscope)
- Validated software for **online motion correction** and **automated signal extraction**

RESULTS & CONCLUSION

- We can record activity from **200-300 synapses** across a dendritic tree at **>200 Hz**
- …and **nearly 1000 synapses** at **>100 Hz,** with excellent SNR
- Our methods enable the study of **how input patterns across the dendritic tree relate to a neuron's output** in awake, behaving mice

I. Visualizing Synaptic Inputs

iGluSnFR4
(Learn more at: PSTR196.02, Mon. Oct 7, 9–10a.m.)

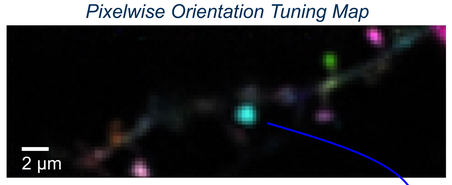


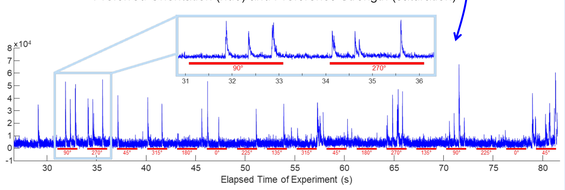
- Fluorescent protein **glutamate indicator**
- Visualizes **synaptic transmission** with high sensitivity

IN VIVO CHARACTERIZATION

- Synapses labeled with iGluSnFR4 in primary visual cortex show **distinct orientation tuning**

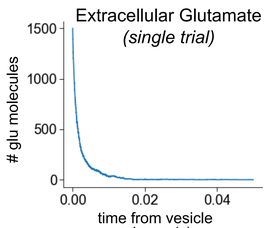
Pixelwise Orientation Tuning Map

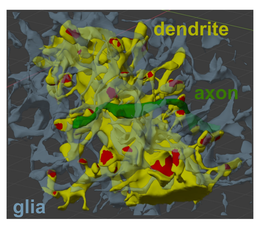


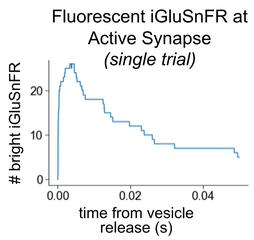


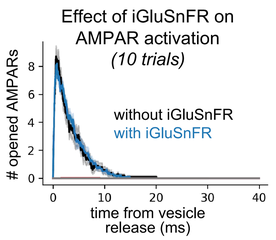
EFFECTS OF GLUTAMATE INDICATORS

- Use Monte Carlo Cell (MCell) to **simulate synaptic response** to vesicle release with and without iGluSnFR (Bartol et al., 2015)





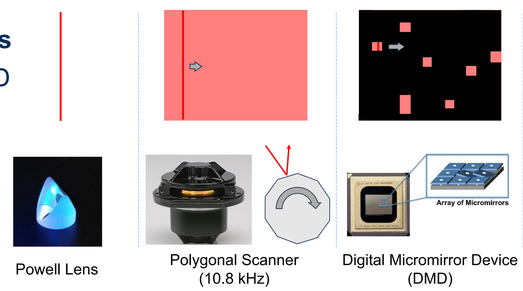




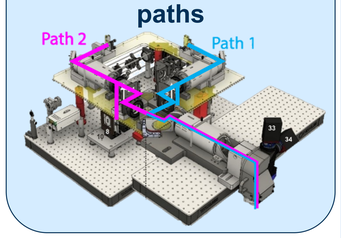
II. Recording Many Sparse Sources Simultaneously at High-Speed

SLAP2 MICROSCOPE

- Microscope that can **simultaneously record from 1000s of synapses**
- Flexibly illumination in 3D
- Raster scanning as well as **high-speed "integration scanning"**
- **Remote focusing** for rapid z-scanning

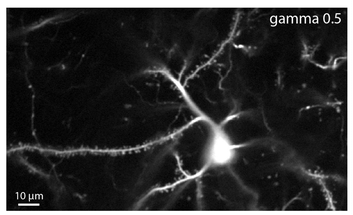


Scan engine is implemented in **two simultaneously-recorded paths**



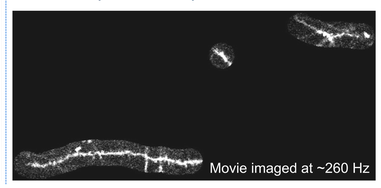
Raster Scanning Mode

1 DMD row is opened at a time for conventional raster scans



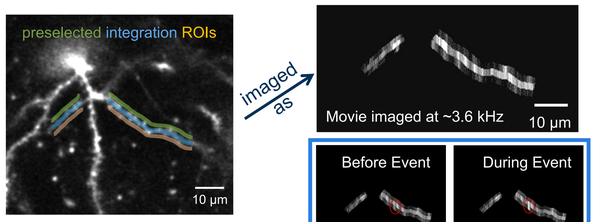
Multi-ROI Scanning Mode

Arbitrary sets of pixels can be raster scanned w/ **no per-ROI access time cost** to **efficiently image sparse structures**
frame period ∝ # pixels in a column

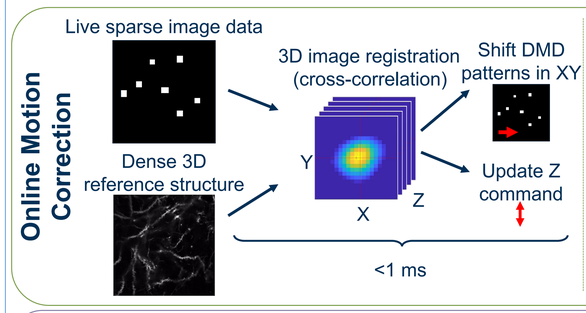


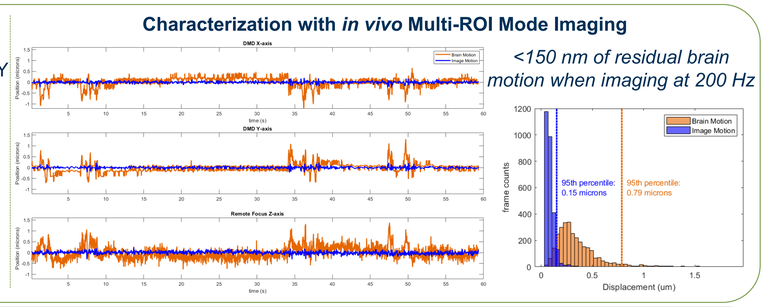
Integration Scanning Mode

Multiple DMD rows are opened at a time, **integrating fluorescence over each column** and **trading off resolution for speed**
frame period ∝ # ROIs in a column

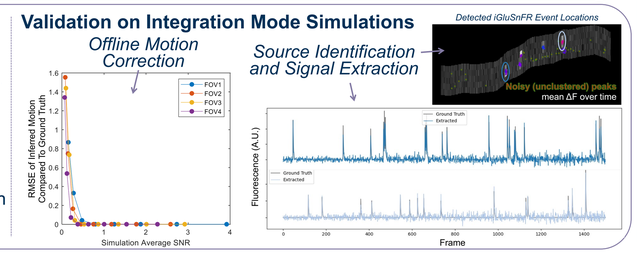


SOFTWARE FOR SLAP2 IMAGING





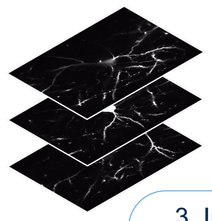
<150 nm of residual brain motion when imaging at 200 Hz



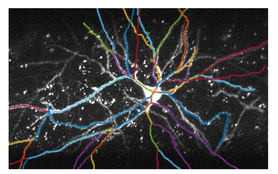
III. Measuring Synaptic Inputs Across the Dendritic Arbor

IMAGING DENDRITES ON SLAP2

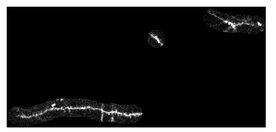
1. Collect SLAP2 reference volume



2. Trace neuron's dendrites

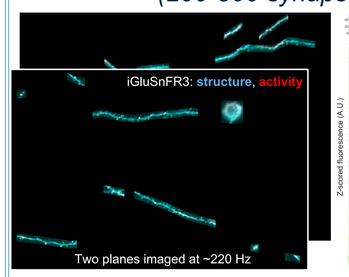


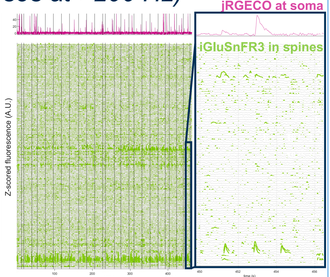
3. Image selected dendrites on SLAP2



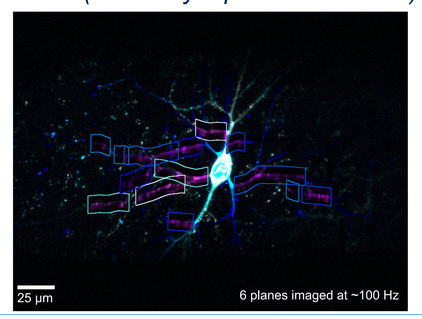
SLAP2 DENDRITIC IMAGING DATA

Imaging Mode 1: Dual-plane multi-ROI
(200-300 synapses at >200 Hz)





Imaging Mode 2: Volumetric integration mode
(~1000 synapses at >100 Hz)



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[1] Aggarwal, A. et al. Glutamate indicators with improved activation kinetics and localization for imaging synaptic transmission. Nat Methods 20, 925–934 (2023).
[2] Bartol, T. M. et al. Computational reconstitution of spine calcium transients from individual proteins. *Front. Synaptic Neurosci.* **7**, (2015).

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